CLAIMS

WE CLAIM:

1. A cell culture composition comprising pluripotent cells and an inhibitor of at least one component of the gamma-secretase complex.
2. The cell culture composition of Claim 1, wherein the pluripotent cells are human cells.
3. The cell culture composition of Claim 2, wherein the human pluripotent cells are selected from the group consisting of human embryonic stem cells, human inner cell mass (ICM)/epiblast cells, human primitive ectoderm cells, and human primordial germ cells.
4. The cell culture composition of Claim 3, wherein the human cells are human embryonic stem cells.
5. The cell culture composition of Claim 1, wherein the inhibitor of at least one component of the gamma-secretase complex is selected from the group consisting of non-transition state analogues, transition state analogs, helical peptides containing α-aminoisobutyric acid, Fenchylamine Sulfonamide compounds, NSAIDs, and benzodiazepines.
6. The cell culture composition of Claim 1, wherein the inhibitor comprises DAPT.
7. The cell culture composition of Claim 1, wherein the inhibitor comprises a transition state analog selected from the group consisting of III-31-C, L-685,458, and a substrate-based difluroketone peptidomimetic.
8. The cell culture composition of Claim 7, wherein the substrate-based difluroketone peptidomimetic is DFK-167.
9. The cell culture composition of Claim 1, wherein the cells are stabilized in a pluripotent state for at least 10 passages.
10. The cell culture composition of Claim 9, wherein the pluripotent state is determined by expression of SSEA4 and Notch1 in at least approximately 60% of the cells.
11. The cell culture composition of Claim 1, wherein less than approximately 20% of the cells express HNF4alpha after approximately 10 passages.

12. The cell culture composition of Claim 1, wherein the inhibitor of at least one component of the gamma-secretase complex is expressed from a feeder cell layer.

13. The cell culture composition of Claim 12, wherein the feeder cell layer is genetically engineered to express the inhibitor.

14. The cell culture composition of Claim 1, wherein the inhibitor of at least one component of the gamma-secretase complex inhibits Notch signaling in the pluripotent cells.

15. A cell culture composition comprising pluripotent cells and an inhibitor of Notch signaling.

16. The cell culture composition of Claim 15, wherein the pluripotent cells are human cells.

17. The cell culture composition of Claim 16, wherein the human pluripotent cells are selected from the group consisting of human embryonic stem cells, human inner cell mass (ICM)/epiblast cells, human primitive ectoderm cells, and human primordial germ cells.

18. The cell culture composition of Claim 17, wherein the human cells are human embryonic stem cells.

19. The cell culture composition of Claim 15, wherein the inhibitor of Notch signaling is selected from the group consisting of a gamma secretase inhibitor, and a dominant negative Notch protein.

20. The cell culture composition of Claim 19, wherein the dominant negative Notch protein comprises an extracellular domain of one or more Notch proteins or a portion thereof.

21. The cell culture composition of Claim 15, wherein the cells are stabilized in a pluripotent state for at least 10 passages.

22. The cell culture composition of Claim 21, wherein the pluripotent state is determined by expression of SSEA4 and Notch1 in at least approximately 60% of the cells.

23. The cell culture composition of Claim 15, wherein less than approximately 20% of the cells express HNF4alpha after approximately 10 passages.

24. The cell culture composition of Claim 15, wherein the inhibitor of Notch signaling is expressed from a feeder cell layer.

25. The cell culture composition of Claim 24, wherein the feeder cell layer is genetically engineered to express the inhibitor.

26. A cell culture composition comprising pluripotent cells and an activator of Notch signaling.

27. The cell culture composition of Claim 26, wherein the pluripotent cells are human cells.

28. The cell culture composition of Claim 27, wherein the pluripotent cells are selected from the group consisting of human embryonic stem cells, human inner cell mass (ICM)/epiblast cells, human primitive ectoderm cells, and human primordial germ cells.

29. The cell culture composition of Claim 28, wherein the human cells are human embryonic stem cells.

30. The cell culture composition of Claim 26, wherein the activator is a ligand selected from the group consisting of Jagged-1, Jagged-2, Jagged-3, Serrate, any member of the Jagged/Serrate protein family, Delta, Delta-like-1, Delta-like-3, Delta-like-4, Delta-like homolog-1 (DLK1); any member of the Delta protein family; and any portion of any of these proteins.

31. The cell culture composition of Claim 26, wherein a majority of the cells are differentiated after culture with the activator.

32. The cell culture composition of Claim 26, wherein the activator is expressed from a feeder cell layer.

33. The cell culture composition of Claim 32, wherein the feeder cell layer is genetically engineered to express the activator.

34. A method of differentiating or stabilizing human pluripotent cells, wherein said method comprises:

a. providing human pluripotent cells that express one or more Notch proteins,

b. providing an activator or inhibitor of at least one of the one or more Notch proteins on the pluripotent cells; and

c. contacting the human pluripotent cells with the activator or inhibitor

to thereby differentiate or stabilize the human pluripotent cells.

35. A method of stabilizing human embryonic stem cells in a pluripotent state, wherein the cells express one or more Notch proteins, wherein said method

comprises providing an inhibitor of Notch signaling to thereby stabilize the cells.

36. A method of stabilizing human pluripotent cells, comprising
    a. providing a human feeder layer wherein the feeder layer expresses an inhibitor of Notch signaling, wherein the inhibitor of Notch signaling is selected from the group consisting of a gamma-secretase inhibitor, and a dominant negative Notch protein; and
    b. contacting the human pluripotent cells with the human feeder layer in a culture medium

    to thereby stabilize the human pluripotent cells in a pluripotent state.

37. The method of Claim 36, wherein the dominant negative Notch protein comprises an extracellular domain of one or more Notch proteins or a portion thereof.

38. The method of Claim 37, wherein the feeder layer is genetically engineered to express the inhibitor of Notch signaling.

39. The method of Claim 36, wherein the expression of the Notch inhibitor is induced by the addition of a compound to the culture medium.

40. A method of controlling the differentiation of human pluripotent cells, comprising
    a. providing a human feeder layer wherein the feeder layer expresses an activator of Notch signaling; and
    b. contacting the human pluripotent cells with the human feeder layer in a culture medium

    to thereby differentiate the human pluripotent cells.

41. The method of Claim 40, wherein the pluripotent cells differentiate into neural cells.

42. The method of Claim 40, wherein the Notch activator is selected from the group consisting of Jagged-1, Jagged-2, Jagged-3, Serrate, any member of the Jagged/Serrate protein family, Delta, Delta-like-1, Delta-like-3, Delta-like-4, Delta-like homolog-1 (DLK1); any member of the Delta protein family; and any portion of any of these proteins.

43. The method of Claim 40, wherein the pluripotent cells are selected from the group consisting of human embryonic stem cells, human inner cell mass

(ICM)/epiblast cells, human primitive ectoderm cells, and human primordial germ cells.

44. The method of Claim 43, wherein the cells are human embryonic stem cells.

45. The method of Claim 40, wherein the expression of the Notch activator is induced by the addition of a compound to the culture medium.

46. A method of stabilizing a pluripotent cell culture, comprising:

a. providing a pluripotent cell culture; and

b. contacting the pluripotent cell culture with an inhibitor of at least one component of the gamma-secretase complex

to thereby stabilize the pluripotent cell culture.

47. The method of Claim 46, wherein the pluripotent cells are human cells.

48. The method of Claim 47, wherein the pluripotent cells are selected from the group consisting of human embryonic stem cells, human inner cell mass (ICM)/epiblast cells, human primitive ectoderm cells, and human primordial germ cells.

49. The method of Claim 48, wherein the human cells are human embryonic stem cells.

50. The method of Claim 46, wherein the inhibitor of at least one component of the gamma-secretase complex is selected from the group consisting of non-transition state analogues, transition state analogs, helical peptides containing α-aminoisobutyric acid, Fenchylamine Sulfonamide compounds, NSAIDs, and benzodiazepines.

51. The method of Claim 50, wherein the inhibitor comprises DAPT.

52. The method of Claim 50, wherein the inhibitor comprises a transition state analog selected from the group consisting of III-31-C, L-685,458, and a substrate-based difluroketone peptidomimetic.

53. The method of Claim 52, wherein the substrate-based difluroketone peptidomimetic is DFK-167.

54. The method of Claim 50, wherein the inhibitor comprises DAPT.

55. The method of Claim 46, wherein the cells are stabilized in a pluripotent state for at least 10 passages.

56. The method of Claim 55, wherein the pluripotent state is determined by expression of SSEA4 and Notch1 in at least approximately 60% of the cells.

57. The method of Claim 46, wherein less than approximately 20% of the cells express HNF4alpha after approximately 10 passages.

58. The method of Claim 46, wherein the inhibitor is expressed from a feeder cell layer.

59. The method of Claim 58, wherein the feeder cell layer is genetically engineered to express the inhibitor.

60. The method of Claim 46, wherein the inhibitor of at least one component of the gamma-secretase complex inhibits Notch signaling in the pluripotent cells.